AGRICULTURAL IMPACT ON BROWN HARE: LC-MS/MS KIDNEY MULTIRESIDUAL PESTICIDES ANALYSES

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Abstract

By the dramatic increase in the areas under the agricultural crops in Serbia with the intensive use of pesticides and the decrease in areas under fodder crops the possibility of qualitative nutrition for hares is decreased parallel with the change of their typical habitats. Hares feed on grass, weeds, various agricultural crops, vegetable plants, buds, fresh trees bark and grains. They provide the sufficient amount of water through succulent plants so that they almost need no water. That is why the aim of our study was to determine the 30 pesticides content by validated multyresidual LC-MS/MS method. Based on the LC-MS/MS analysis of brown hare kidney samples eight pesticides outof 30 were detected. The detections were very low, except one sample, which contained oxamil, carbendazim and cymoxanil residues in the concentrations above EU MRLs, namely 0.446, 0.071 and 0.711 mg/kg, respectively. In the other sample oxamyl residue was over the EU MRLs.

Introduction

According to the Food and Agriculture Organization's article [1], 11.0% of Earth's 13.4 billion hectares of land are used for crop cultivation. The plant agriculture leaves one of humanity's biggest ecological footprints and hence has major implications for wild-animal suffering. The crop cultivation plausibly reduces populations of large number of animals [2]. The increasing human populations are associated with greater conversion and fragmentation of wild habitats, and more intense hunting pressure on remaining wildlife stocks. Animals are a part of many agricultural systems. Wild animals can help to manage pest populations and contribute to biodiversity [3]. FAO‘s concern is that wildlife species are increasingly at risk from the expansion and intensification of agricultural production. The agricultural impact could lead to habitat loss and environmental damage due to pesticides being dispersed into the environment [4]. By the dramatic increase in the areas under the agricultural crops in Serbia with the intensive use of pesticides and the decrease in areas under fodder crops the possibility of qualitative nutrition for hares is decreased parallel with the change of their typical habitats [5-7]. Hares feed on grass, weeds, various agricultural crops, vegetable plants, buds, fresh trees bark and grains. They provide the sufficient amount of water through succulent plants so that they almost need no water [5].

That is why the aim of our study was to determine the 30 pesticides content by validated multyresidual method liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

Experimental

Chemicals

The analitical fungicide standards were manufactured by Dr. Ehrenstorfer GmbH, Germany. As an internal standard carbofuran-D3 (99.7%) was purchased from Pestanal, Fluka (Germany) and was used in the concentration of 1.0 mg/mL of the basic standard in acetonitrile with the dilution up to 10.0 μg/mL. The stock standard solutions were prepared by dissolving an analytical standard in acetonitrile while the working solution i.e. the mixture of the studied pesticides was obtained by mixing and diluting the stock standards with acetonitrile resulting in the final mass concentration of 10.0 μg/mL.

Aparature

For LC analysis, an Agilent 1200 (Agilent Technologies, USA) HPLC system th a binary pump was used. This was equipped with a reversed-phase C18 analytical column of 50×4.6mm and 1,8 μm particle size (Agilent Zorbax Eclipse XDB). The mobile phase was methanol and Milli-Q water with 0.1% formic acid in gradient mode, with the flow rate 0.4 mL/min. For the mass spectrometric analysis, an Agilent 6410 Triple-Quad LC/MS system was applied. Agilent Mass Hunter Data Acquisition, Qualitative Analysis and Quantitative Analysis software were used for method development and data acquisition.

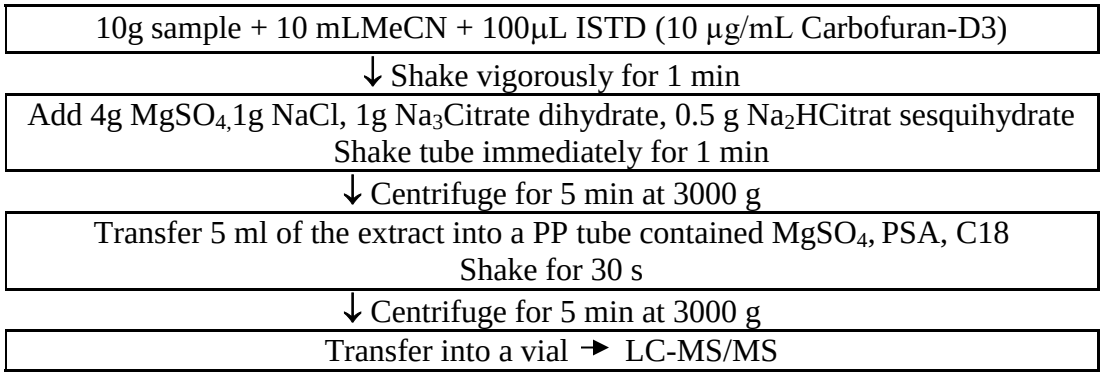
Validation

Within the validation the recoveries of extraction, detection limits (LOD), quantification limits (LOQ) and linearity with carbofuran-D3 addition as an internal standard (IS) were determined according to SANTE/11945/2015 [8]. The LOD was determined as the lowest concentration giving a response of five times the average baseline. The ratio signal/noise in the obtained chromatograms for the LOD was calculated by MassHunter Qualitative Software. The linearity was checked using matrix matched calibration (MMC) at the concentrations of 5.0, 10.0, 25.0, 50.0 and 100.0 ng/mL.The recovery was checked by enriching 10 g of a blank sample with the mixture of pesticide standard of 10 μg/mL in the amount of 100 and 50 μL (final mass concentration 0.10 and 0.05 mg/kg) and with the mixture of pesticide standard of 1 μg/mL in the amount of 100 μL (final mass concentration 0.01 mg/kg) with the addition of the internal standard carbofuran-D3.

Samples and fungicide extraction

Brown hare were collected from the agricultural areas from Vojvodina region. The kidneys of ten animals were immediately collected and put in dark plastic bags. All the samples were put in the freezer until they were analyzed by QuEChERS method (Fig. 1).

Figure 1. QuEChERS extraction of fungicide residues



Results and discussion

The obtained LC-MS/MS TIC chromatogram of pesticide standards (Figure 1) and LC-MS/MS chromatogram of sample number six chromatogram (Figure 2) are shown below.

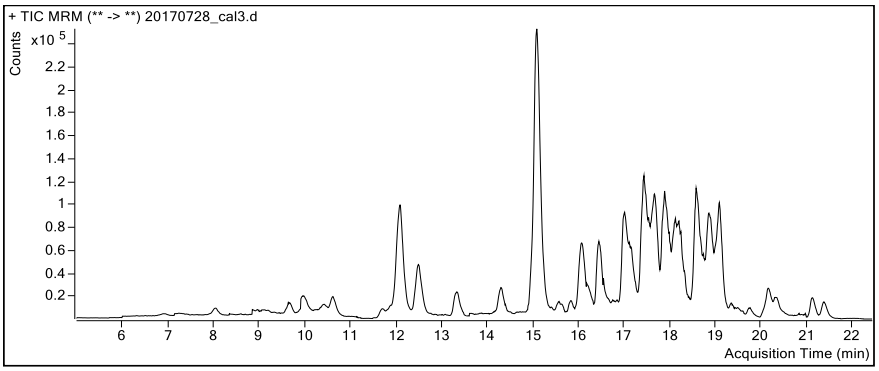


Figure 1. TIC chromatogram of pesticide standards

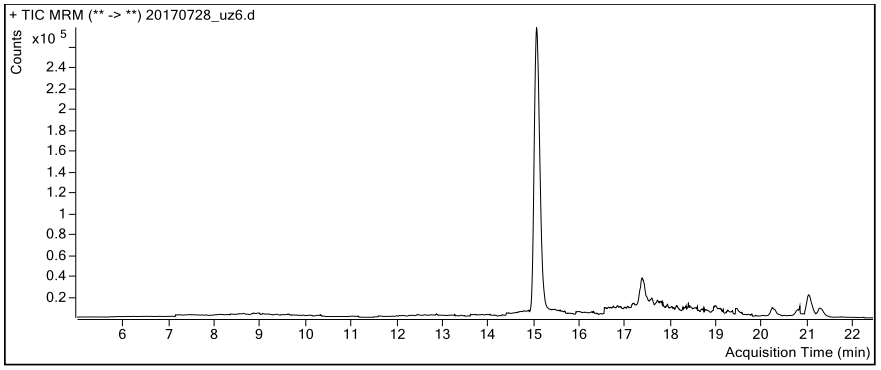


Figure 2. TIC chromatogram of sample number six

According to the obtained LC-MS/MS results, some pesticides were detected (mg/kg) in analysed brown hare kidney samples (Table 1).

No	Omethoate	Oxamyl	Carbendazim	Thiamethoxam	Cymoxanil	Thiacloprid	Phosmet	Dimethomorph
1	<LOQ	0.003	<LOQ	0.004	0.006	0.005	<LOQ	<LOQ
2	<LOQ	**0.446**	**0.071**	0.061	**0.711**	0.008	<LOQ	0.005
3	<LOQ	0.006	0.005	0.004	0.005	<LOQ	0.007	<LOQ
4	<LOQ	0.006	<LOQ	<LOQ	0.002	<LOQ	0.007	<LOQ
5	0.003	**0.012**	0.005	0.004	0.024	<LOQ	0.007	<LOQ
6	0.003	0.007	<LOQ	<LOQ	0.013	0.005	<LOQ	0.003
7	0.003	0.004	0.004	<LOQ	0.011	<LOQ	<LOQ	<LOQ
8	0.003	0.003	<LOQ	<LOQ	0.004	0.005	<LOQ	<LOQ
9	0.003	0.008	0.005	<LOQ	0.012	<LOQ	<LOQ	<LOQ
10	0.003	0.003	<LOQ	0.004	0.009	0.005	0.007	0.003

Conclusion

Based on the LC-MS/MS analysis of brown hare kidney samples eight pesticides outof 30 were detected. The detections were very low, except in sample number 2, which contained oxamil, carbendazim and cymoxanil residues in the concentrations above EU MRLs [9], namely 0.446, 0.071 and 0.711 mg/kg, respectively. In sample number 5 oxamyl residue was over the EU MRLs [9].

Acknowledgements

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